

Human Milk Oligosaccharides: 2'-fucosyllactose (2'-FL) and Lacto-N-neotetraose (LNnT) in infant formula

Y. Vandenplas (1), B. Berger (2), V. P. Carnielli (3), J. Ksiazzyk (4), H. Lagström (5), M Sanchez Luna (6), N. Migacheva (7), J.M. Mosselmans (8), J.C. Picaud (9), M. Possner (10), A.Singhal (11), M.Wabitsch (12)

1. KidZ Health Castle, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium
2. Department of Gastro-Intestinal Health, Nestlé Health Sciences, Nestlé Research, Nestec Ltd, Lausanne, Switzerland,
3. Neonatal Pediatrics, Polytechnic University of Marche, Ancona, Italy
4. The Children's Memorial Health Institute, Dept. of Paediatrics, Nutrition and Metabolic Diseases, Warsaw, Poland
5. Department of Public Health, University of Turku and Turku University Hospital, Turku, Finland
6. Neonatology Division. Complutense University. Research Institute University Hospital Gregorio Marañón, Madrid, Spain.
7. Department of Pediatrics, Samara State Medical University, Samara, Russia.
8. Citadelle For Life, Brussels, Belgium
9. Neonatology, Croix-Rousse Hospital, Lyon and CarMen unit, INSERM U1060, INRA U197, Claude Bernard University, Lyon 1, Pierre-Bénite, France
10. Nestlé Nutrition Institute
11. Paediatric Nutrition, Great Ormond Street, UCL, Institute of Child Health, London, United Kingdom
12. Ulm University Hospital, Department of Paediatrics and Adolescent Medicine, Division of Paediatric Endocrinology and Diabetes, Centre for Hormonal Disorders in Children and Adolescents, Ulm, Germany

Corresponding Author: Prof. Dr. Yvan Vandenplas. KidZ Health Castle, UZ Brussel, Vrije Universiteit Brussel, Laarbeeklaan, 101, 1090, Brussels, Belgium.

Tel: +32 (0)24775794; Fax +32 (0)24775783; E-mail: yvan.vandenplas@uzbrussel.be

Disclosures:

Y Vandeplass has participated as a clinical investigator, and/or advisory board member, and/or consultant, and/or speaker for Abbott Nutrition, Biocodex, Danone, Nestle Health Science, Nestle Nutrition Institute, Nutricia, Mead Johnson, United Pharmaceuticals. B. Berger is employee of Nestec Ltd, a subsidiary company of the Nestlé group. V. Carnielli has participated as an advisory board member for Nestle Nutrition Institute. J. Ksiazek was a speaker for Danone, Nutricia, Nestle, Fresenius Kabi and participated in the meetings of Nestle Nutrition Institute. H. Lagström has participated as advisory board member, consultant and speaker for Nestlé Nutrition Institute and Nestlé Finland. M. Sanchez Luna has participated as a clinical investigator, and/or advisory board member, and/or consultant, and/or speaker for Abbvie, Dräger, Nestle Nutrition Institute, Linde Healthcare. N. Migacheva declares no conflict of interest. J.M. Mosselmans is Advisory Board moderator for Nestle Nutrition Institute. J.C. Picaud has participated as a clinical investigator, and/or advisory board member, and/or speaker for Nestle Nutrition Institute, Modilac France, Bledina France and Nestlé Health Science. M. Possner is employed by Nestlé Nutrition Institute. A. Singhal has participated as a clinical investigator, and/or advisory board member, and/or speaker for Abbott Nutrition, Wyeth Nutrition, Nestle Health Science, Nestle Nutrition Institute, Danone and Phillips. M. Wabitsch has participated as advisory board member for Nestle Nutrition Institute

Abstract**Introduction**

The authors reviewed the published evidence on the presence of oligosaccharides in human milk (HMO) and their benefits in in vitro and in vivo studies, as well as to evaluate published data on the addition of some of HMOs to infant formula. .

Method

PubMed was searched from inception to April 2018

Results

The presence of HMOs is a dynamic process, determined by many factors such as duration of lactation, environmental and genetic factors. HMOs may support immune function development and provide protection against infectious diseases directly through the interaction of the gut epithelial cells or indirectly through the modulation of the gut microbiota, including the stimulation of the bifidobacteria. Only a few of the many HMOs can be made industrially, and are added to infant formula. They have been shown to be safe and well tolerated, including normal growth and a trend towards health benefit.

Conclusions

HMOs are one of the major differences between cow's milk and human milk , and evidence indicates that these components do have a health promoting benefit. The addition of one or two of these components to infant formula is safe, and brings infant formula closer to human milk.

Key words: breast feeding; formula feeding; human milk oligosaccharide; 2'-fucosyllactose; Lacto-N-neotetraose; microbiota; bifidobacteria

Introduction

Breast milk is the natural and ideal food for babies, providing the energy and nutrients that every infant needs during the first four to six months of life in the correct quality and amount. Infants who are breastfed for shorter periods or are not breastfed suffer more infectious diseases, such as gastroenteritis and acute otitis media, more immune mediated diseases, have a lower intelligent quotient (IQ) and are likely to have a higher risk of overweight and type 2 diabetes in later life [1,2]. However, any breastfeeding is beneficial. In a pooled analysis of 24 studies from the USA and Europe, for example, any form of breastfeeding was found to be protective for acute otitis media in the first two years of life, but exclusive breastfeeding for the first six months was associated with the greatest protection [3].

The composition of breast milk is unique. Aside from nutrients for the infant's healthy growth and development, it contains thousands bioactive substances [4], including human milk oligosaccharides (HMOs) [5]. HMOs are non-digestible carbohydrates [6]. Although they have little nutritional value for the infant, HMOs are the third largest solid component in human milk after lactose and lipids [7,8]. More than 200 free oligosaccharide structures have so far been identified from human milk samples [9]. Compared to human milk, oligosaccharide concentrations in the milk of farm animals such as cows, goats and sheep are 100–1000-fold lower. In fact, these unique complex carbohydrate structures in human milk are virtually absent in cow milk or any other farmed animal milk and their variety is much lower [10].

Human Milk Oligosaccharides (HMOs)

Around 1900, infant mortality rate (deaths in the first year of life per 1000 live births) in Europe was very high at up to and above 20% [11, 12]. Mortality was especially high in non-breastfed infants and was 7 times greater in bottle fed than breastfed infants [11,13]. It was around this time that differences in stool bacterial composition were discovered between breastfed and formula-fed infants and both breastfeeding and the resulting gut microbiota were linked to the better health of the infants [11,13]. In the 1930s, oligosaccharides were identified as the most important bifidogenic factor in human milk [11]. The most abundant oligosaccharides in human milk were discovered in 1954. However, it was only recently that scientists and industry were able to produce the first oligosaccharides structurally identical to those in human milk [14]. It is important to note that HMOs resist cold and heat, and are not affected by pasteurization and freeze-drying [15,16].

The amount of HMOs is 20-25 g/L in colostrum and 10-15 gram per liter (g/L) in mature milk, or 1.5-2.3g/100 kcal assuming an energy density of human milk of 64 kcal/100ml [13,17]. Three major HMO categories are present in breast milk: i) fucosylated neutral HMOs (35-50%), ii) sialylated acidic HMOs (12-14%) and iii) non-fucosylated neutral HMOs (42-55%) [18,19]. Neutral HMOs account for more than 75% of the total HMOs in human breast milk. 2'-fucosyllactose (2'-FL) is part of the fucosylated, while Lacto-N-neotetraose (LNnT) is part of the non-fucosylated neutral HMOs. In women who are "secretors", 2'-FL is by far the most abundant HMO and constitutes nearly 30% of all HMOs.

All HMOs are synthesized in the mammary gland [20]. The amount and composition of HMOs vary between women and over the course of lactation. HMO concentration is higher during early stages of lactation and decreases gradually over time [21,22,23]. A recent study could not confirm the observation that the content of HMOs is also higher after a term than preterm delivery (Kunz et al. 2017). The most extreme intra individual variation in HMO fucosylation and are based on the maternal secretor and Lewis blood group status [13,20,24]. Both the fucosyltransferase 2/FUT2 or secretor gene and the fucosyltransferase 3/FUT3 or Lewis gene are expressed in glandular epithelia. The secretor (Se) gene encodes for the α 1-2-fucosyltransferase enzyme (FUT2) which is necessary for the synthesis of 2'-FL and other Fucosyl-HMOs and is expressed in the lactating mammary gland. The milk of secretor (Se+) women is therefore characterized by an abundance of α 1-2-fucosylated HMOs, especially 2'FL [6,13,24]. Non-secretors, by contrast, lack the FUT2 enzyme and therefore their milk does not contain 2'FL and other α 1-2-fucosylated HMOs, or is in only minimal amounts [6,25]. The absence of 2'FL and other α 1-2-fucosylated HMOs explains the lower total amount of HMOs in "non-secretor" milk [20]. For example, a recent study found approximately 35% to 45% less total HMOs in the milk of non-secretor Lewis-positive women than in the milk of Lewis-positive secretor women [20]. The acidic HMOs do not depend on secretor status [20].

Based on the expression of FUT2 and FUT3, breast milk can be assigned to one of four groups (Table 1) [13]:

Group 1: Secretors, Lewis-positive, (Se+Le+) (FUT2 active, FUT3 active)

Group 2: Non-secretors, Lewis-positive, (Se-Le+) (FUT2 inactive, FUT3 active)

Group 3: Secretors, Lewis-negative (Se+Le-) (FUT2 active, FUT3 inactive)

Group 4: Non-secretors, Lewis-negative (Se-Le-) (FUT2 inactive, FUT3 inactive)

About 80% of the European and American women are secretors [26]. About 70% of the populations are Lewis-positive secretors (Se+Le+) and around 5-10% are Lewis-negative secretors (Se+Le-) [27].

However, other factors also influence HMO synthesis. A recent study showed that HMO content and profiles vary geographically, even when secretor and Lewis blood group genes were taken into account [20,28]. Findings on HMO concentrations over time of lactation and clusters based on 2'-FL concentrations suggest that LNnT and Lacto-N-Tetraose (LNT) are 'co-regulated' with the FUT2 dependent 2'-FL concentration, with LNnT showing a positive and LNT a negative relation to the amount of 2'-FL [6]. Mothers' milk with low levels of 2'-FL also contains low levels of LNnT but high levels of LNT [6]. The clinical impact of these findings still needs to be unraveled.

The European Union (EU) considers two HMOs, 2'-FL and LNnT, novel foods (Commission Implemented Regulation (EU) 2017/2470). Today, the USA's FDA considers 3 HMOs to be Generally Regarded As Safe (GRAS notice no 650). On June 29, 2015, the European Food Safety Authority (EFSA) based on the scientific and technical information provided, concluded that 2-FL is safe for infants up to one year of age when added to infant and follow-on formulae, in combination with LNnT, at concentrations up to 1.2 g/L of 2'-FL and up to 0.6 g/L of LNnT, at a ratio of 2:1 in the reconstituted formulae. 2'-FL is safe for young children (older than one year of age) when added to follow-on and young-child formulae, at concentrations up to 1.2 g/L of 2'-FL (alone or in combination with LNnT, at concentrations up to 0.6 g/L, at a ratio of 2:1)" (EFSA-Q-2015-00052, EFSA Journal 2015).

Health benefit of Human Milk Oligosaccharides (HMO)

Secretor milk (due to its high levels of 2'FL and other Fucosyl-HMOs) may have advantages for the baby because it, more effectively promotes an early high *Bifidobacteria*-dominated gut microbiota [29] and provides better protection against specific diarrheal diseases [30] than non-secretor milk.

Several studies have documented beneficial effects of HMOs, including modification of the intestinal microbiota, anti-adhesive antimicrobial effects, modulation of intestinal epithelial cell response, effects on immune development and on brain development.

Preclinical and observational studies

Modification of the intestinal microbiota

Data from *in vitro* studies have shown that HMOs promote the growth of certain, but not all, bifidobacteria [13]. *Bifidobacterium longum subsp. infantis* (*B. infantis*) grows well on HMOs (including 2'-FL) as the sole source of carbohydrates [31,32,33,34]. Compared to *B. infantis*, *B. bifidum* grows slightly slower on HMOs [33]. Recent literature showed in strains from other bifidobacterial species that the metabolic capacity to utilize HMOs is not restricted to *B. infantis* [35,36,37].

The fact that HMOs are a preferred substrate for *B. infantis* and other bifidobacteria strains may reduce the nutrients available for potentially harmful bacteria and keep their growth under control. In addition, *B. infantis* produces short-chain fatty acids (SCFA), which help create an environment favoring the growth of commensal bacteria instead of potential pathogens [38].

An *in vitro* study evaluated HMOs' utilization by *Enterobacteriaceae*, which has been linked to the onset of necrotizing enterocolitis (NEC) in preterm infants [39]. The study showed that none of the *Enterobacteriaceae* strains grow on 2'-FL, 6-sialyllactose (6'-SL), and LNnT, whereas several *Enterobacteriaceae* strains, including pathogens, grew well on galacto-oligosaccharides (GOS) [39].

Observational studies showed that 2'Fucosyl-HMOs are associated with bifidobacteria dominated early gut microbiota in breast-fed infants [29,35,40]. The influence of secretor status and breastfeeding on gut microbiota composition persists up to two to three years [40].

Anti-adhesive antimicrobial

Many viruses, bacterial pathogens or toxins need to adhere to mucosal surfaces to colonize or invade the host and cause disease [13,41,42]. Some HMOs are structurally similar to the intestinal epithelial cell surface glycan receptors and serve as decoy receptors to prevent pathogen binding and enhance pathogen clearance [13]. This unique beneficial effect of HMOs is highly dependent on their structure.

Evidence for an anti-adhesive effect of specific HMOs comes from *in vitro* and *ex vivo* studies. For instance, Ruiz-Palacios *et al* demonstrated that human milk oligosaccharides inhibited *Campylobacter jejuni* (*C. jejuni*) adherence to epithelial cells *in vitro* [43], one of the major causes of bacterial diarrhea worldwide. A second study conducted by the same group confirmed that fucosylated human milk oligosaccharides inhibit campylobacter colonization of human intestinal mucosa *ex vivo* [43]. Yu *et al* tested the ability of 2'-FL to inhibit *C. jejuni* infection of the intestinal epithelium and *C. jejuni*-associated mucosal inflammation [44]. In an *in vitro* model, 2'-FL attenuated 80% of *C. jejuni* invasion ($p < 0.05$) and decreased the release of

mucosal pro-inflammatory signals. In a mouse model, ingestion of 2'-FL reduced *C. jejuni* colonization by 80%, weight loss by 5%, intestinal inflammation (shown by histologic features), and induction of inflammatory signaling molecules ($p < 0.05$) [44].

In infants, observations from a prospective study conducted by Morrow *et al* suggested a beneficial effect of α 1-2-fucosylated HMO on reducing episodes of *C. jejuni*-associated diarrhea [30]. In Mexican breast-fed infants, *Campylobacter* diarrhea occurred less often in those infants whose mother's milk contained a high percentage of milk oligosaccharides of 2'FL. Lower percentage total 2-linked fucosylated oligosaccharides as a had a dose-dependent association with higher rates of moderate-to-severe diarrhea of all causes. The association between milk oligosaccharides measured during the first months and diarrhea in breast-fed infants persisted through the course of lactation but not after cessation of breastfeeding [30].

Other observational studies of breast-fed infants also suggested beneficial effects of fucosyl-HMOs in breast milk. They showed that fucosyl-HMOs in breast milk are related to lower morbidity in Gambian infants at four months of age [45] and fewer respiratory and enteric problems in US infants at 3 months of age [46].

The influence of 2'-FL and 6'-SL on adhesion of *E. coli* and *Salmonella fytis* to Caco-2 cells was tested with positive results for *E. coli* but not for *Salmonella* [47];

HMOs have also been suggested to possibly protect against important systemic infections of the newborn. For instance, LNnT reduces *Streptococcus (S.) pneumoniae* load in lungs in a rabbit model [48]. A clinical study with infants older than 6 months, however, could not achieve a

reduction in the colonization of the oropharynx with *S. pneumoniae* through a synthetic LNnT-supplemented infant formula [49].

HMOs may function as an alternative substrate to modify a group B *Streptococcus* component in a manner that impairs growth kinetics [50]. There is a unique antibacterial role for HMOs against this leading neonatal pathogen [50].

There is increasing evidence that HMOs could reduce infant mortality and morbidity in preterm infants, for example by shaping a favorable gut microbiome protecting against NEC, candidiasis, and several other immune-related diseases [51]. In support of this, lower concentration of the HMO disialyllacto-N-tetraose (DSLNT) was shown to predict the risk of NEC in preterm infants. This finding has been demonstrated by a recent multicentre clinical cohort study including 200 mothers and their very low birthweight infants who were predominantly human milk-fed [52]. DSLNT concentrations were significantly lower in almost all milk samples in NEC cases compared with controls, and its abundance could identify NEC cases prior to onset, i.e. DSLNT content in breast milk is a potential non-invasive marker to identify infants at risk of developing NEC, and screen high-risk donor milk. Beneficial effects on NEC have also been reported for 2'-FL. Good *et al* demonstrated that 2'FL attenuates the severity of the experimental NEC by enhancing mesenteric perfusion in the neonatal intestine on an experimental mouse model of NEC [53].

Modulators of intestinal epithelial cell response

HMOs are able to reduce cell growth, induce differentiation, apoptosis and maturation, and increase the barrier function *in vitro* [54,55,56,57] Intestinal health and intestinal barrier function constitute the first defense line in innate immunity.

Zehra *et al* demonstrated that the HMOs 6'-sialyllactose and 2'-FL modulate human epithelial cell responses related to allergic disease in different ways [58]. 6'-Sialyllactose inhibited chemokine (IL-8 and CCL20) release from T-84 and HT-29 cells stimulated with antigen-antibody complex TNF- α or PGE-2; an effect that was PPAR γ dependent and associated with decreased activity of the transcription factors AP-1 and NF κ B. In contrast, 2'-FL selectively inhibited CCL20 release in response to the antigen antibody complex in PPAR γ dependent manner. These findings reinforce the concept that structurally different oligosaccharides have distinct biological activities and identifies, and for the first time, that the HMOs, 6'-SL, and 2'-FL, modulate human epithelial cell responses related to allergic disease. This encourages further investigation of the therapeutic potential of specific HMOs in food allergy [58].

Immune modulators

Among the multiple functions of HMOs, immunomodulation is one of the most remarkable [59]. HMOs directly affect intestinal epithelial cells and modulate their gene expression, which leads to changes in cell surface glycans and other cell responses. HMOs modulate lymphocyte cytokine production, potentially leading to a more balanced TH1/TH2 response.

An increasing number of *in vitro* studies suggest that HMOs not only affect the infant's immune system indirectly by changing gut microbiota but also directly modulate immune responses by affecting immune cell populations and cytokine secretion [5]. HMOs may either act locally on cells of the mucosa-associated lymphoid tissues or on a systemic level [13].

Dietary HMOs were more effective than non-human prebiotic oligosaccharides in altering systemic and gastrointestinal immune cells in pigs [60]. These altered immune cell populations may mediate the effects of dietary HMOs on rotavirus infection susceptibility [60]. Daily oral treatment with 2'-FL attenuated food allergy symptoms in a mouse model by induction of IL-10+ T-regulatory cells and indirect stabilization of mast cells [61].

In vitro studies have shown that 2'-FL directly inhibits LPS-mediated inflammation during enterotoxigenic *E. coli* (ETEC) invasion of T84 (modeling mature) and H4 (modeling immature) intestinal epithelial cells through attenuation of CD14 induction [62]. CD14 expression mediates lipopolysaccharide-TLR4 (toll-like receptor 4) stimulation of portions of the 'macrophage migration inhibitory factors' inflammatory pathway via suppressors of cytokine signalling 2/signal transducer and activator of transcription 3/NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells).

In an animal model, early life provision for a period of 6 weeks of 1 % authentic HMOs delayed and suppressed Type 1 diabetes development in non-obese diabetic mice and reduced the development of severe pancreatic insulinitis in later life [63]. In a murine influenza vaccination model dietary 2'-FL improved both humoral and cellular immune responses to vaccination in mice, enhancing vaccine specific delayed-type hypersensitivity responses accompanied by increased serum levels of vaccine-specific immunoglobulin proliferation. Vaccine-specific CD4+ and CD8+ T-cells as well as interferon-gamma production were significantly increased in spleen cells of mice receiving 2'-FL leading to the conclusion that dietary intervention with 2'-FL improved both humoral and cellular immune responses to vaccination in mice [64].

Brain development

Metabolic products of HMOs such as sialic acid promote brain development, neuronal transmission and synaptogenesis. HMOs provide sialic acid as potentially essential nutrients for brain development and cognition [65,66]. Application of L-fucose and 2'-FL increases the potentiation of the population spike amplitude (POP-spike) and the field excitatory postsynaptic potential (fEPSP) after tetanization of the Schaffer collaterals of the rat hippocampus [67]. Dietary 2'-FL interferes with cognitive domains and improves learning and memory in rodents [68]. HMOs, 3'Sialyllactose and 6'Sialyllactose, support normal microbial communities and behavioral responses during stressor exposure, potentially through effects on the gut microbiota-brain axis [69].

Improved gut adaptation after resection

Patients with short bowel syndrome require parental nutrition and may require frequent treatment with antibiotics that modify intestinal microbiota and have an adverse effect on gastrointestinal function [70]. The hypothesis that 2'-FL contributes to the adaptive response after intestinal resection was confirmed on the basis of a murine model of intestinal adaptation. Modulating of gut microbiota following intestinal resection improved the outcome of short bowel syndrome in an experimental setting. Supplementation with 2'-FL increased weight gain following ileo-cecal resection and promoted histological changes of gut mucosa suitable for adaptation [71]. (

Clinical studies with 2'-FL

One prospective, randomized, controlled study tested the tolerance and safety in relation of growth of an infant formula containing 2'-FL (0.2 g/L or 1.0 g/L) in combination with galacto-

oligosaccharides (2.2 g/L or 1.4 g/L) in healthy full-term infants from 28 sites across USA [72]. No differences in growth parameters and adverse events were found between the infants fed the formula with 2'-FL from enrolment (0-5 days of age) up to the age of four months compared to infants fed a control formula containing only galacto-oligosaccharides and between both formula-fed groups and the breast-fed reference group. This study was the first publication showing that growth of infants consuming a formula containing 2'-FL was similar to that of human milk fed infants [72]. Both formulas with 2'-FL and galacto-oligosaccharides were well tolerated and did not influence stool frequency or consistency.

The effects of feeding formulas supplemented with 2'-FL on biomarkers of immune function was investigated in a subgroup of this study population (Goehring et al. 2016). Infants fed formulas with 2'-FL and galacto-oligosaccharides had 29-83% lower concentrations of plasma inflammatory cytokines and tumor necrosis factor α (TNF- α) than infants fed the control formula with galacto-oligosaccharides only [73]. There were no differences in plasma inflammatory cytokines and TNF- α between infants fed formulas with 2'-FL and galacto-oligosaccharides and infants breast fed. These findings indicate that supplementation of infant formula with 2'-FL supports aspects of immune development and regulation similar to that of breast-fed infants; while supplementation with galacto-oligosaccharides alone does not [73].

Another prospective, randomized, controlled study tested the gastrointestinal tolerance of an infant formula containing 2'-FL (0.2 g/L) in combination with fructo-oligosaccharides (2 g/L) compared to a control formula without oligosaccharides [74]. The formula with 2'-FL and fructo-oligosaccharides fed from less than eight days of age for approximately one month was well tolerated; stool consistency, anthropometric data and frequency of feedings with spitting -

up/vomiting and was similar to that of infants given formula without oligosaccharides or to infants breast-fed [74].

Puccio *et al.* conducted the first clinical trial with an infant formula supplemented with two HMOs [75]. In this prospective, randomized, controlled multicenter study, healthy term infants received a formula with 2'-FL and LNnT or the same formula without HMOs from enrolment at ≤ 14 days of age to age six months and for at least four months as the exclusive diet [75]. The formula with 2'-FL and LNnT was well-tolerated and supported age-appropriate growth. Gastrointestinal symptoms (flatulence, spitting up and vomiting) were similar between the groups. Infants receiving formula with 2'-FL and LNnT had significantly softer stools and fewer episodes of night-time wake-ups at age two months, and infants born by caesarian section also had a lower incidence of colic at four months of age. Puccio *et al.* also analyzed the incidence of different health outcomes as secondary outcomes. Infants fed the formula with 2'-FL and LNnT compared to infants fed the formula without HMOs had significantly fewer parental reports of bronchitis (at 4, 6 and 12 months), reduced incidence of lower respiratory tract infections (through 12 months), reduced use of antipyretics (through 4 months) and reduced use of antibiotics (through 6 and 12 months) with protective effects that continued after the 6 months intervention period) [75].

In the same trial, infants fed the formula with 2'-FL and LNnT developed a gut microbiota that was closer to the microbiota observed in breastfed infants [76]. At three months of age, the stool microbiota was characterized by an increased quantity of beneficial Bifidobacteria and decreased abundances of taxa with potentially pathogenic members. Moreover, the supplementation of an infant formula with these two HMOs promoted the growth of a distinct fecal bacteria

community, typical of breastfed infants and showing a very high density of bacteria. Formula fed infants carrying this fecal community type had a two times decreased risk of requiring antibiotics during the first year of life [76]. Therefore, this study suggests that the association between consuming formula with 2'-FL and LNnT and lower parent-reported morbidity and medication use may be linked to gut microbiota community types [76].

Conclusion

HMOs act as soluble decoy receptors that block the attachment of specific viral, bacterial or protozoan parasite pathogens to epithelial cell surface sugars, which may in turn help prevent infectious diseases in the gut, respiratory and urinary tracts. In addition, HMOs alter host epithelial and immune cell responses with potential benefits for the neonate, beyond protection against infectious diseases.

Although the functions of HMOs have been known for many years, it was not possible to synthesize them on an industrial scale until recently. With the goal of imitating their effect, non-human milk oligosaccharides, mainly fructo and galacto-oligosaccharides have been added to infant formula. In recent years it has, to a certain extent, become technically possible to add 2'-FL and LNnT to infant formula.

The addition of one HMO, namely 2'-FL, is a step forward to bring formula feeding closer to the gold standard: breastmilk. No adverse effects have been reported for 2'-FL and in vitro and animal studies have shown benefits of supplementation of infant formula with 2'-FL. Clinical studies in infants show a normal growth pattern and normal defecation, and suggest clinical benefit. It can therefore be concluded that 2'-FL is a safe supplementation of infant formula.

References

1. ESPGHAN Committee on Nutrition; Agostoni, C.; Braegger, C.; Decsi, T.; Kolacek, S.; Koletzko, B.; Michaelsen, K.F.; Mihatsch, W.; Moreno, L.A.; Puntis, J.; Shamir, R.; Szajewska, H.; Turck, D.; van Goudoever, J.; Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **2009**, *49*, 112-125.
2. Victora, C.G.; Bahl, R.; Barros, A.J.; França, G.V.; Horton, S.; Krasevec, J.; Murch, S.; Sankar, M.J.; Walker, N.; Rollins, N.C.; Lancet Breastfeeding Series Group. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet* **2016**, *387*, 475-490.
3. Bowatte, G.; Tham, R.; Allen, K.J.; Tan, D.J.; Lau, M.; Dai, X.; Lodge, C.J.; Breastfeeding and childhood acute otitis media: a systematic review and meta-analysis. *Acta Paediatr.* **2015**, *104*, 85-95.
4. Mosca, F.; Gianni, M.L. Human milk: composition and health benefits. *Pediatr Med Chir.* **2017**, *39*, 155.
5. Donovan, S.M.; Comstock, S.S. Milk oligosaccharides influence neonatal mucosal and systemic immunity. *Ann. Nutr. Metab.* **2016**, *69* Suppl 2, 42-51.
6. Sprenger, N.; Lee, L.Y.; De Castro, C.A.; Steenhout, P.; Thakkar, S.K. Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS One.* **2017**, *12*, e0171814.
7. Ballard, O.; Morrow, A.L. Human milk composition: nutrients and bioactive factors. *Pediatr. Clin. North Am.* **2013**, *60*, 49-74.

8. Austin, S.; De Castro, C.A.; Bénet, T.; Hou, Y.; Sun, H.; Thakkar, S.K.; Vinyes-Pares, G.; Zhang, Y.; Wang, P. Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrients*. **2016**, 8, 8(6). pii: E346
9. Ruhaak, L.R.; Lebrilla, C.B. Advances in analysis of human milk oligosaccharides. *Adv. Nutr.* **2012**, 3, 406S-414S.
10. Urashima, T.; Taufik, E.; Fukuda, K.; Asakuma, S. Recent advances in studies on milk oligosaccharides of cows and other domestic farm animals. *Biosci Biotechnol Biochem.* **2013**, 77, 455-466.
11. Kunz, C. Historical aspects of human milk oligosaccharides. *Adv. Nutr.* **2012**, 3, 430S-439S
12. Elwood, J.H. Infant mortality in Belfast and Dublin--1900-1969. *Ir J Med Sci.* **1973**, 142, 166-173.
13. Bode, L. Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology.* **2012**, 22, 1147-1162.
14. Petschacher, B.; Nidetzky, B. Biotechnological production of fucosylated human milk oligosaccharides: Prokaryotic fucosyltransferases and their use in biocatalytic cascades or whole cell conversion systems. *J. Biotechnol.* **2016**, 235, 61-83.
15. Hahn, W.H.; Kim, J.; Song, S.; Park, S.; Kang NM. The human milk oligosaccharides are not affected by pasteurization and freeze-drying. *J. Matern. Fetal Neonatal Med.* **2017**, 6, 1-7.
16. Daniels, B.; Coutoudis, A.; Autran, C.; Amundson Mansen, K.; Israel-Ballard, K.; Bode, L. The effect of simulated flash heating pasteurisation and Holder pasteurisation on human milk oligosaccharides. *Paediatr Int Child Health.* **2017**, 37, 204-209

17. Zivkovic, A.M.; German, J.B.; Lebrilla, C.B.; Mills, D.A. Human milk glycobioime and its impact on the infant gastrointestinal microbiota. *Proc. Natl. Acad. Sci. USA.* **2011**, 108 Suppl 1, 4653-4658
18. Smilowitz, J.T.; Lebrilla, C.B.; Mills, D.A.; German, J.B.; Freeman, S.L. Breast milk oligosaccharides: structure-function relationships in the neonate. *Ann Rev Nutr.* **2014**, 34, 143-169.
19. [Van Niekerk, E.](#); [Autran, C.A.](#); [Nel, D.G.](#); [Kirsten, G.F.](#); [Blaauw, R.](#); [Bode, L.](#) Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. *J Nutr.* **2014**, 144, 1227-1233.
20. Akkerman, R.; Faas, M.M.; de Vos, P. Non-digestible carbohydrates in infant formula as substitution for human milk oligosaccharide functions: Effects on microbiota and gut maturation. *Crit Rev Food Sci Nutr.* **2018**, 15, 1-12.
21. Xu, G.; Davis, J.C.; Goonatileke, E.; Smilowitz, J.T.; German, J.B.; Lebrilla, C.B. Absolute quantitation of human milk oligosaccharides reveals phenotypic variations during lactation. *J Nutr.* **2017**, 147, 117-124.
22. Chaturvedi, P.; Warren, C.D.; Altaye, M.; Morrow, A.L.; Ruiz-Palacios, G.; Pickering, L.K.; Newburg, D.S. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology.* **2001**, 11, 365-372
23. Thurl, S.; Munzert, M.; Henker, J.; Boehm, G.; Muller-Werner, B.; Jelinek, J. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. *Br J Nutr.* **2010**, 104, 1261-1271

24. Kunz, C.; Rudloff, S. Compositional analysis and metabolism of human milk oligosaccharides in infants. *Nestle Nutr Inst Workshop Ser.* **2017**, *88*, 137-147.
25. Jantscher-Krenn, E.; Bode, L. Human milk oligosaccharides and their potential benefits for the breast-fed neonate. *Minerva Pediatr.* **2012**, *64*, 83-99.
26. Goehring, K.C.; Kennedy, A.D.; Prieto, P.A.; Buck, R.H. Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. *PLoS One.* **2014**, *9*, e101692.
27. Rudloff, S.; Kunz, C. Milk oligosaccharides and metabolism in infants. *Adv Nutr.* **2012**, *3*, 398S-405S.
28. McGuire, M.K.; Meehan, C.L.; McGuire, M.A.; Williams, J.E.; Foster, J.; Sellen, D.W.; Kamau-Mbuthia, E.W.; Kamundia, E.W.; Mbugua, S.; Moore, S.E.; Prentice, A.M.; Kvist, L.J.; Otoo, G.E.; Brooker, S.L.; Price, W.J.; Shafii, B.; Placek, C.; Lackey, K.A.; Robertson, B.; Manzano, S.; Ruíz, L.; Rodríguez, J.M.; Pareja, R.G.; Bode, L.. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr.* **2017**, *105*, 1086-1100.
29. Lewis, Z.T.; Totten, S.M.; Smilowitz, J.T.; Popovic, M.; Parker, E.; Lemay, D.G.; Van Tassell, M.L.; Miller, M.J.; Jin, Y.S.; German, J.B.; Lebrilla, C.B.; Mills, D.A. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome.* **2015**, *3*, 13.
30. Morrow, A.L.; Ruiz-Palacios, G.M.; Altaye, M.; Jiang, X.; Guerrero, M.L.; Meinzen-Derr, J.K.; Farkas, T.; Chaturvedi, P.; Pickering, L.K.; Newburg, D.S. Human milk oligosaccharide blood group epitopes and innate immune protection against campylobacter and calicivirus diarrhea in breastfed infants. *Adv Exp Med Biol.* **2004**, *554*, 443-446.

31. Lo Cascio, R.G.; Ninonuevo, M.R.; Freeman, S.L.; Sela, D.A.; Grimm, R.; Lebrilla, C.B.; Mills, D.A.; German, J.B. Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. *J Agric Food Chem.* **2007**, *55*, 8914-8919.
32. Marcobal, A.; Barboza, M.; Froehlich, J.W.; Block, D.E.; German, J.B.; Lebrilla, C.B.; Mills, D.A. Consumption of human milk oligosaccharides by gut-related microbes. *J Agric Food Chem.* **2010**, *58*, 5334-5340.
33. Asakuma, S.; Hatakeyama, E.; Urashima, T.; Yoshida, E.; Katayama, T.; Yamamoto, K.; Kumagai, H.; Ashida, H.; Hirose, J.; Kitaoka, M. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. *J Biol Chem.* **2011**, *286*, 34583-34592.
34. [Bunesova, V.](#); [Lacroix, C.](#); [Schwab, C.](#) Fucosyllactose and L-fucose utilization of infant *Bifidobacterium longum* and *Bifidobacterium kashiwanohense*. *BMC Microbiol.* **2016**, *16*, 248.
35. Matsuki, T.; Yahagi, K.; Mori, H.; Matsumoto, H.; Hara, T.; Tajima, S.; Ogawa, E.; Kodama, H.; Yamamoto, K.; Yamada, T.; Matsumoto, S.; Kurokawa, K. A key genetic factor for fucosyllactose utilization affects infant gut microbiota development. *Nat Commun* **2016**, *7*, 11939.
36. James, K.; Motherway, M.O.; Bottacini, F.; van Sinderen, D. *Bifidobacterium breve* UCC2003 metabolises the human milk oligosaccharides lacto-N-tetraose and lacto-N-neo-tetraose through overlapping, yet distinct pathways. *Sci Rep.* **2016**, *6*, 38560.
37. Garrido, D.; Ruiz-Moyano, S.; Kirmiz, N.; Davis, J.C.; Totten, S.M.; Lemay, D.G.; Ugalde, J.A.; German, J.B.; Lebrilla, C.B.; Mills, D.A. A novel gene cluster allows preferential utilization of fucosylated milk oligosaccharides in *Bifidobacterium longum* subsp. *longum* SC596. *Sci Rep.* **2016**, *6*, 35045.

38. Gibson, G.R.; Wang, X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J Appl Bacteriol.* **1994**, *77*, 412-420
39. Hoeflinger, J.L.; Davis, S.R.; Chow, J.; Miller, M.J. In vitro impact of human milk oligosaccharides on Enterobacteriaceae growth. *J Agric Food Chem.* **2015**, *63*, 3295-3302.
40. Smith-Brown, P.; Morrison, M.; Krause, L.; Davies, P.S. Mothers secretor status affects development of childrens microbiota composition and function: a pilot study. *PLoS One.* **2016**, *11*, e0161211.
41. Bode L. The functional biology of human milk oligosaccharides. *Early Hum Dev.* **2015**, *9*, 619-622.
42. Hu, L.; Crawford, S.E.; Czako, R.; Cortes-Penfield, N.W.; Smith, D.F.; Le Pendu, J.; Estes, M.K.; Prasad, B.V. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature.* **2012**, *485*, :256-259.
43. Ruiz-Palacios, G.M.; Cervantes, L.E.; Ramos, P.; Chavez-Munguia, B.; Newburg, D.S. *Campylobacter jejuni* binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. *J Biol Chem.* **2003**, *278*, 14112-14120.
44. Yu, Z.T.; Nanthakumar, N.N.; Newburg, D.S. The human milk oligosaccharide 2'-fucosyllactose quenches *Campylobacter jejuni*-induced inflammation in human epithelial cells HEp-2 and HT-29 and in mouse intestinal mucosa. *J Nutr.* **2016**, *146*, 1980-1990.
45. Davis, J.C.; Lewis, Z.T.; Krishnan, S.; Bernstein, R.M.; Moore, S.E.; Prentice, A.M.; Mills, D.A.; Lebrilla, C.B.; Zivkovic, A.M. Growth and morbidity of Gambian infants are influenced by maternal milk oligosaccharides and infant gut microbiota. *Sci Rep.* **2017**, *7*, 40466.

46. Stepans, M.B.; Wilhelm, S.L.; Hertzog, M.; Rodehorst, T.K.; Blaney, S.; Clemens, B.; Polak, J.J.; Newburg, D.S. Early consumption of human milk oligosaccharides is inversely related to subsequent risk of respiratory and enteric disease in infants. *Breastfeed Med.* **2006**, *1*, 207-215.
47. Facinelli, B.; Marini, E.; Magi, G.; Zampini, L.; Santoro, L.; Catassi, C.; Monachesi, C.; Gabrielli, O.; Coppa GV. Breast milk oligosaccharides: effects of 2'-fucosyllactose and 6'-sialyllactose on the adhesion of *Escherichia coli* and *Salmonella fytis* to Caco-2 cells. *J Matern Fetal Neonatal Med.* **2018**, 1-3.
48. Idänpään-Heikkilä, I.; Simon, P.M.; Zopf, D.; Vullo, T.; Cahill, P.; Sokol, K.; Tuomanen, E. Oligosaccharides interfere with the establishment and progression of experimental pneumococcal pneumonia. *J Infect Dis.* **1997**, *176*, 704-712.
49. Prieto, P.A. In vitro and clinical experiences with a human milk oligosaccharide, Lacto-N-neoTetraose, and Fructooligosaccharides. *Foods Food Ingredients J Jpn.* **2005**, *210*, 1018-1030.
50. Lin, A.E.; Autran, C.A.; Szyszka, A., Escajadillo, T.; Huang, M.; Godula, K.; Prudden, A.R.; Boons, G.J.; Lewis, A.L.; Doran, K.S.; Nizet, V.; Bode, L. Human milk oligosaccharides inhibit growth of group B *Streptococcus*. *J Biol Chem.* **2017**, *292*, 11243-11249.
51. Moukarzel, S.; Bode, L. Human milk oligosaccharides and the preterm infant: a journey in sickness and in health. *Clin Perinatol.* **2017**, *44*, 193-207.
52. Autran, C.A.; Kellman, B.P.; Kim, J.H.; Asztalos, E.; Blood, A.B.; Spence, E.C.; Patel, A.L.; Hou, J.; Lewis, N.E.; Bode, L. Human milk oligosaccharide composition predict risk of necrotizing enterocolitis in preterm infants. *Gut.* **2018**, *67*, 1064-1070.

53. Good, M.; Sodhi, C.P.; Yamaguchi, Y.; Jia, H.; Lu, P.; Fulton, W.B.; Martin, L.Y.; Prindle, T.; Nino, D.F.; Zhou, Q.; Ma, C.; Ozolek, J.A.; Buck, R.H.; Goehring, K.C.; Hackam, D.J. The human milk oligosaccharide 2'-fucosyllactose attenuates the severity of experimental NEC by enhancing mesenteric perfusion in the neonatal intestine. *Br J Nutr.* **2016**, *116*, 1175-1187.
54. Kuntz, S.; Rudloff, S.; Kunz, C. Oligosaccharides from human milk influence growth-related characteristics of intestinally transformed and non-transformed intestinal cells. *Br J Nutr.* **2008**, *99*, :462-471.
55. Kuntz, S.; Kunz, C.; Rudloff, S. Oligosaccharides from human milk induce growth arrest via G2/M by influencing growth-related cell cycle genes in intestinal epithelial cells. *Br J Nutr.* **2009**, *101*, 1306
56. Holscher, H.D.; Davis, S.R.; Tappenden, K.A. Human milk oligosaccharides influence maturation of human intestinal Caco-2Bbe and HT-29 cell lines. *J Nutr.* **2014**, *144*, 586-591.
57. Holscher, H.D.; Bode, L.; Tappenden, K.A. Human milk oligosaccharides influence intestinal epithelial cell maturation in vitro. *J Pediatr Gastroenterol Nutr.* **2017**, *64*, 296-301.
58. Zehra, S.; Khambati, I.; Vierhout, M.; Mian, M.F.; Buck, R.; Forsythe, P. Human milk oligosaccharides attenuate antigen-antibody complex induced chemokine release from human intestinal epithelial cell lines. *J Food Sci.* **2018**, *83*, 499-508.
59. Kulinich, A.; Liu, L. Human milk oligosaccharides: The role in the fine-tuning of innate immune responses. *Carbohydr Res.* **2016**, *432*, 62-70.
60. Comstock, S.S.; Li, M.; Wang, M.; Monaco, M.H.; Kuhlenschmidt, T.B., Kuhlenschmidt, M.S.; Donovan, S.M. Dietary human milk oligosaccharides but not prebiotic

oligosaccharides increase circulating natural killer cell and mesenteric lymph node memory T-cell populations in non-infected and rotavirus-infected neonatal piglets. *J Nutr.* **2017**, *147*:1041.

61. Castillo-Courtade, L.; Han, S.; Lee, S.; Mian, F.M.; Buck, R.; Forsythe, P. Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy.* **2015**, *70*, 1091-1102. .

62. He, Y.; Liu, S.; Leone, S.; Newburg, D.S. Human colostrum oligosaccharides modulate major immunologic pathways of immature human intestine. *Mucosal Immunol.* **2014**, *7*, 1326-1339.

63. Xiao, L.; Van't Land, B.; Engen, P.A.; Naqib, A.; Green, S.J.; Nato, A.; Leusink-Muis, T.; Garssen, J.; Keshavarzian A.;Stahl, B.; Folkerts, G.; Human milk oligosaccharides protect against the development of autoimmune diabetes in NOD-mice. *Sci Rep.* **2018**, *8*, 3829.

64. Xiao, L.; Leusink-Muis, T.; Kettelarij, N.; van Ark, I.; Blijenberg, B.; Heslen, N.A.; Stahl, B.; Overbeek, S.A.; Garssen, J.; Folkerts, G.; Van't Land, B. Human milk oligosaccharide 2'-Fucosyllactose improves innate and adaptive immunity in an influenza-specific murine vaccination model. *Front Immunol.* 2018, ;9, :452.on 2018.

65. Bienenstock, J.; Buck, R.H.; Linke, H.; Forsythe, P.; Stanisiz, A.M.; Kunze, W.A. Fucosylated but not sialylated milk oligosaccharides diminish colon motor contractions. *PLoS One.* **2013**, *2*, 8(10):e76236.

66. Jacobi, S.K.; Yatsunenko, T.; Li, D.; Dasgupta, S.; Yu, R.K.; Berg, B.M.; Chichlowski, M.; Odle, J. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *J Nutr.* **2016**, *146*, 200-208.

67. Matthies, H.; Staak, S.; Krug, M. Fucose and fucosyllactose enhance in-vitro hippocampal long-term potentiation. *Brain Res.* **1996**, *725*, :276-280.
68. Vázquez, E.; Barranco, A.; Ramírez, M.; Gruart, A.; Delgado-García, J.M.; Martínez-Lara, E.; Blanco, S.; Martín, M.J.; Castanys, E.; Buck, R.; Prieto, P.; Rueda, R. Effects of a human milk oligosaccharide, 2'-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents. *J Nutr Biochem.* **2015**, *26*, 455-465..
69. Tarr, A.J.; Galley, J.D.; Fisher, S.E.; Chichlowski, M.; Berg, B.M.; Bailey, M.T. The prebiotics 3'Sialyllactose and 6'Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut-brain axis. *Brain Behav Immun.* **2015**, *50*, 166-177.
70. Sommovilla, J.; Zhou, Y.; Sun, R.C.; Choi, P.M.; Diaz-Miron, J.; Shaikh, N.; Sodergren, E.; Warner, B.B.; Weinstock, G.M.; Tarr, P.; Warner, B.W. Small bowel resection induces long-term changes in the enteric microbiota of mice. *J Gastrointest Surg.* **2015**, *19*, 56-64.
71. Mezzoff, E.A.; Hawkins, J.A.; Ollberding, N.J.; Karns, R.; Morrow, A.L.; Helmrath, M.A. The human milk oligosaccharide 2'-fucosyllactose augments the adaptive response to extensive intestinal resection. *Am J Physiol Gastrointest Liver Physiol.* 2016;310, G427-438.
72. Marriage, B.J.; Buck, R.H.; Goehring, K.C.; Oliver, J.S.; Williams, J.A. Infants fed a lower calorie formula with 2'-fucosyllactose (2'-FL2'-FL) show growth and 2'-FL2'-FL uptake like breast-fed infants. *J Pediatr Gastroenterol Nutr.* **2015**, *61*, 649-658.
73. Goehring, K.C.; Marriage, B.J.; Oliver, J.S.; Wilder, J.A.; Barrett, E.G.; Buck, R.H. Similar to those who are breastfed, infants fed a formula containing 2'-fucosyllactose have lower inflammatory cytokines in a randomized ocnrolled trial. *J Nutr.* **2016**, *146*, 2559-2566..

74. Kajzer, J.; Oliver, J.; Marriage, B. Gastrointestinal tolerance of formula supplemented with oligosaccharides. *The FASEB Journal*. **2016**, 30(1 Supplement), 671.4
75. Puccio, G.; Alliet, P.; Cajozzo, C.; Janssens, E.; Corsello, G.; Sprenger, N.; Wernimont, S.; Egli, D.; Gosoni, L.; Steenhout, P. Effects of infant formula with human milk oligosaccharides on growth and morbidity: a randomized multicenter trial. *J Pediatr Gastroenterol Nutr*. **2017**, 64, 624-631.
76. Berger (paper submitted)

Table 1. Diversity of HMO based on genetic background of the mother

Gene	Lewis gene +	Lewis gene -
Secretor gene +	Lewis positive secretors	Lewis negative secretors
	Secrete all HMOs	Secrete 2'-FL2'-FL, 3'FL, LNFP-I, LNFP-III
Secretor gene -	Lewis positive non-secretors	Lewis negative non-secretors
	Secrete 3'FL, LNFP-II and LNFP III	Secrete 3'FL, LNFP-III and LNFP-V